IMPACT ASSESSMENT REPORT

Prepared For The

BUREAU OF LAND MANAGEMENT
As Part Of The
California Commercial and
Sports Fish Oil Toxicity Study

Contract AA851-CTO-74 ,2910

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CONTENTS

LIST OF TABLES

IMPACT ASSESSMENT REPORT

INTRODUCTION

Mollusks, crustaceans and fishes represent the majority of California's living marine resources (Frey 1971). For this reason, we have restricted our review of the hydrocarbon effects literature to these three broad taxonomic groups. Relatively few species of California marine organisms of commercial or sport importance have been studied in terms of the lethal or sublethal effects of hydrocarbon exposure. From literature reviewed for this report, limited information concerning hydrocarbon effects was available for only four California species: California mussel, dungeness crab, Pacific herring and northern anchovy.

Most literature concerning the effects of hydrocarbon exposure on marine organisms has dealt with species having little commercial or sport value. This is unfortunate, however, since these economically valuable marine resources may be impacted by OCS oil and gas development. Because literature dealing with the effects of hydrocarbon exposure on California marine mollusks, crustaceans, and fishes is generally lacking, we have reviewed all available literature on these groups from California and other geographical areas.

Many of the initial studies dealing with oil toxicity concentrated on adult life history stages. It soon became apparent, however, that all life history stages needed to be examined since hydrocarbon exposure was likely to affect each stage differently. In particularly, the embryonic and larval stages of marine organisms are generally considered to be most sensitive to oil pollution (Hyland and Schneider, 1976). Furthermore, juvenile or adult stages can generally-avoid or emigrate from contaminated areas, whereas early life history stages are less capable of doing so (Sharp et al, 1979).

Survival of the early life history stages (i.e. eggs and larvae) of marine organisms is normally very low under the best of environmental conditions. Accordingly, it is important to develop an understanding of the effects of hydrocarbon exposure on these highly susceptible stages. Because small variations in the annual mortality rate experienced by the early life history stages of marine organisms are the cause of year-to-year fluctuations in recruitment (Cushing, 1975), it is possible that chronic or acute oil exposure may have a pronounced effect upon the size of commercial or sport populations.

Toxicity testing has generally been subdivided into acute effects and chronic effects, although no precise definition of acute has been made. Generally, acute testing has referred to short-term oil exposures with death as the only measured parameter, although the units of measurement vary from time to concentration to dose. For discussion purposes acute toxicity here will refer to petroleum hydrocarbon exposures of 96 hrs or less with death as the measure of effect (Table 1).

Chronic exposures then cover all other situations. Chronic implies a long-term, and is here defined as exposure for longer than 96 hours. Mortality, usually tied to units of time or amount of petroleum hydrocarbon present or both, is again the most common parameter measured. However, with the longer exposure .time of chronic situations comes an increased opportunity to measure sublethal effects. The 96 hours of an acute test is generally too short to allow quantification of anything but mortality, and hydrocarbon concentrations large enough to give meaningful death rates are rarely small enough to cause subtle effects.

Sublethal effects are a critical component of petroleum hydrocarbon exposure. While large spills attract, the greatest attention, it is widely accepted that long-term exposures are ecologically more damaging. Any toxicant that sufficiently affects behavior, movement, feeding ability, respiration, or any pattern integral to the continuing success of a population or community can eliminate that entity.

Table 1. Toxicity bioassays with juvenile/adult fishes.

Citation	Species	Hydrocarbon	LC50 24	LC50 48	LC50 96	TL_m 24	TL_m 48	TL_m 96
		MOLLUSKS						
Nunes and Benville (1978)	Tapes semidecussata	Cook Inlet Crude (CICO) WSF		96	7.35 ppm			
		CRUSTACEANS						
Broderson et al. (1977)	coonstripe shrimp	Cook Inlet Crude (CICO)						
	Stage I				1.8 ppm			
	Stage II				0.77			
	Stage III				0.35			
	Stage IV				1.9			
	Stage V				0.96			
	Stage VI				0.24			
	kelp shrimp larvae				1.1			
	king crab larvae				1.1			
Wells and Sprague (1976)	American lobster larvae	Venezuelan Crude						
	Stage 1				0.86			
	Stage 3				4.6			
	Stages 3 & 4				4.9			
Rice et al. (1979)	grass shrimp	Cook Inlet Crude (CICO)					0.87	
	bumpy shrimp						1.79	
	kelp shrimp						1.86	
	pink shrimp						4.94	
	king crab						3.69	
	hairy hermit crab						8.45	
	amphipod						>10.58	
	mysi d						>7.98	
							>9.02	
Tatem et al. (1978)	Palaemonetes pugio	No. 2 fuel oil 21°			3.5			
		240			1.9			
		28°			1.2			
		320			1.6			
		Bunker C 21°			2.6			
		24°			3.1			
		32°			2.2			

Table 1 (Cent).

Citation	Species	Hydrocarbon	LC50 24	LC50 48	LC50 96	TL_m 24	TL_m 48	TL_m 96
CRUSTACEANS (Cent)								
Tatem et al. (1978) (cent)	Palaemonetes pugio	Louisiana Crude 21°			>19.8			
		24 "			15.9			
		32°			10.7			
		trimethylbenzene	7.0	5.6	5.4			
		benzene	43.5	35.0	27.0			
		tol uene	9.5	15.5	21.0			
		xyl ene	7.4	8.5	14.0			
		phenol	53.0	11.0	5.8			
Anderson et al. (1974b)	Mysidopsis almyra	Louisiana Crude (LCO)					25.0	
		Kuwait Crude (KCO)					18.0	
		No. 2 fuel oil					1.3	
	Palaemontes pugio	Louisiana Crude (LCO)					62.0	
		Kuwait Crude (KCO)					36.0	
		No. 2 fuel oil					3.4	
	Panaeus aztecus	Louisiana Crude (LCO)					58.0	
		Kuwait Crude (KCO)						
		No. 2 fuel oil					9.4	
Battelle (1973)	dungeness crab	No. 2 fuel oil (undispersed)						4778 (µg/ml)
Brodersen et al. (1977)	scooter shrimp	Cook Inlet Crude (WSF)			5.9			
	kelp shrimp				4.3			
	bumpy shrimp				1.7			
	coonstripe shrimp				7.9			
	king crab				2.0			
Anderson et al. (1980)	Pandalus danae	Prudhoe Bay Crude 12°	2.6					
		7°	3.9					
		11.4°		1.8				
		11.4°			3.8			
		11.4°			5.9			
		12-13°			3.4			

Table 1 (Cent).

Citation	Species	Hydrocarbon	LC50 24	LC50 48	LC50 96	TL_m 24	TL_m 48	TL_m 96
CRUSTACEANS (Cent)								
Anderson et al. 1980) (Cent)	Pandalus danae	12-13°			4.8			
		13.7°		2.2				
		13.7°			4.2			
		13.7°			6.2			
		FISHES						
Anderson et al. (1979b)	Cyprinodon variegates	Louisiana crude (LCO)			19.8 ppm			
		Kuwait crude (KCO)			--			
		No. 2 fuel oil			6.3			
		Bunker C fuel			9.0			
Anderson et al. (1974)	Menidia beryllina	LCO			5.5			
		KC 0			6.6			
		No. 2 fuel oil			3.9			
		Bunker C fuel			2.8			
Moles et al. (1979) " "	dolly varden	Prudhoe Bay crude (PBCO)				2.7		
	arctic grayling	Prudhoe Bay crude (PBCO)				4.4		
	slimy sculpin	Prudhoe Bay crude (PBCO)				6.4		
	threespine stickl eback	Prudhoe Bay crude (PBCO)				>10.5		
	dolly varden	Benzene				11.7		
	arctic grayling	Benzene				14.7		
	slimy sculpin	Benzene				15.4		
	threespine sticklebacks	Benzene				24.8		
Rice et al. (1976)	pink salmon	Cook Inlet Crude (CICO)				4.13	2.92	
	dolly varden	Cook Inlet Crude (CICO)				3.25	2.94	
	cod	Cook Inlet Crude (CICO)				2.48	2.28	
	tubesnout	Cook Inlet Crude (CICO)					1.34	
	pink salmon	No. 2 fuel oil				0.89	0.81	
	dolly varden	No. 2 fuel oil				--	2.29	
	cod	No. 2 fuel oil				4.56	2.93	

Table 1 (Cent).

Citation	Species	Hydrocarbon	LC50 24	LC50 48	LC50 96	TL_m 24	TL_m 48	TL_m 96
FISHES (Cent)								
Hedtke and Puglisi (1980)	flagfish	(WSF) waste crankcase oil (static)			36200 ml/1			
		(WSF) waste crankcase oil (flow-through)			9500 ml/1			
		(OWD) waste crankcase oil (static)			485 ml/1			
		(OWD) waste crankcase oil (flow-through)			83 ml/1			
Kern et al. (1979)	pink salmon	CICO 4°					1.45	
		8°					1.69	
		12°					1.77	
		Toluene 4°					6.41	
		8°					7.63	
		12°					8.09	
		Napthalene 4°					1.37	
		8°					1.84	
		12°					1.24	
Moles (1980)	coho salmon	PBCO			10.38			
		napthalene			3.22			
		toluene			9.30			
Rice et al. (1977)	pink salmon	CICO				4.13	2.9	
		PBCO				1.56	1.6	
		No. 2 fuel oil				0.89	0.8	
Benville and Kern (1977)	striped bass	p-xylene	2.0		2.0			
		ethyl benzene	4.3		4.3			
		benzene	6.9		5.8			
		toluene	7.3		7.3			
		m-xylene	9.2		9.2			
		o-xylene	11.0		11.0			
Thomas and Rice (1979)	pink salmon	toluene				5.38		
		napthalene				0.92		
		CICO				1.73		
		No. 2 fuel oil				0.65		
Meyerhoff (1975)	striped bass	benzene			10.9			

Most acute toxicity testing, and much chronic toxicity testing has been performed under static conditions. Such conditions, however", rarely occur in the real-world. Except for some situations, such as backwaters or largely isolated lagoons, water is constantly exchanged. Failure to do so in the laboratory during chronic exposure testing can seriously weaken the applicability of acquired data to perform real-world projections. A better approximation of true conditions of petroleum hydrocarbon exposure is the use of a flow-through system during testing, in which "new" water is constantly introduced to the system. This duplicates more nearly the real-world situation, and prevents static maintenance problems associated with metabolic waste buildup or reduced oxygen availability from biasing the test.

Oil existing as a slick has clear-cut effects: invertebrates and fish mired in the slick almost always die. However, a great deal of variability exists among oils, and even among batches of the same oil exposed to dissimilar weathering situations in the environment. Much of this variability is in the water soluble fraction (WSF).

The WSF of petroleum hydrocarbons is generally considered to be the most toxic aspect of oil in the environment. Rice et al. (1977) found the toxicity resulted from the chemical properties of the soluble aromatic hydrocarbons.

One approach to studying WSF has been the use of a single petroleum hydrocarbon, known to be a large contributor to the WSF (e.g. naphthalene which is a common component of most crude oils). Use of such single sources allows the toxicant to be defined and measured very accurately, and any effects resulting from exposure of animals to that chemical can be then associated with that chemical specifically. Such applications make utilization of single chemical testing attractive, but, as with any artificial system, some loss of information can also occur. In a real-world situation, not a single chemical, but crude oil or other complex oil compounds are most often spilled. The WSF which animals are usually

exposed to contains a composite of aromatics leached from the slick. Even if a given chemical makes up a large part of the WSF, its toxicity or sublethal effects may not exactly mirror the effects of the WSF. Therefore, the total WSF appears to be the area potential chronic studies can most meaningfully explore.

An important variable that has a profound effect on the utility of information gained from laboratory experiments is the concentration of toxicant administered. The major shortcoming of many studies reviewed in the following pages was that realistic (real-world) concentrations were not utilized in the experimental exposures. In addition, the wide range of levels that were used made comparisons between studies difficult and of limited value. To determine realistic concentrations for experiments, real-world concentrations should be examined.

Environmental levels of petroleum hydrocarbons fall into three general categories: 1) "ambient" levels existing throughout the geographic area under consideration, and displaying some range depending on depth, time of year, and proximity to land; 2) "chronic" levels which are usually localized and indicate the presence of a relatively continuous hydrocarbon input source (e.g. natural oil seep); and 3) "acute" levels which are usually localized and ephemeral indicating the presence of a temporary input source (e.g. oil spill from an accidental discharge).

Hydrocarbon levels in seawater reported for various worldwide geographic areas (Corner and Harris 1968) were variable (Table 2). DeLappe (1980) also recorded a wide range of ambient petroleum hydrocarbon levels (Table 3) that varied with season, depth, and geographic location in the southern California Bight. Even with this high degree of variability, levels were in the nanogram/liter (parts per trillion) range. Chronic levels in the Bight were also low. Payne (personal communication) recorded hydrocarbons in the water column near the active natural oil seeps at Coal Oil Point at 5 ppb. Acute doses of oil released

Table 2. Levels of hydrocarbons in seawater.

Region	Depth	Concentration (ppb) Filter	Particulate	Dissolved	Reference
Outer reaches of Chedabucto Bay (Nova Scotia)	1 m	0.45 µm	5-16[a]	15-90[a]	Levy (1971)
Gotland Deep (Baltic)	20-200 m	Whatman GF/C	0.5-2.3[b]	48-64[b]	Zsolnay (1971)
Baffin Bay (Texas)		0.3 µm	70[c]	180c	Jeffrey (1970)
Cap Ferrat (Mediterranean)	50 m	0.45 µm	-	20.7 -81.9	Coplin & Barbier (1971)
Brest	Surface	0.45 µm	-	137	Barbier et al.
Villefranche	50 m	0.45 µm	-	75	(1973)
Roscoff	Surface	0.45 urn	-	46	"
Open sea off W. Africa	50-4500 m	0.45 µm	-	10-95	"
N.W. Atlantic (Halifax-Bermuda section)	0-3 mm			20.4[d]	Gordon et al. (1974)
	1 m			0.8[d]	"
	5 m			0.4[d]	"
	5 m			Nil	"

a as Bunker C oil equivalents
b as carbon
c unsaturated hydrocarbons
d as Venezuelan oil equivalents

into the environment, such as was the case with the recent IXTOC spill also fell within the high parts per trillion, low parts per billion range.

The design of laboratory experiments that attempt to make environmental predictions from results should incorporate experimental variables that reflect real-world conditions as much as possible. The most critical of these are listed in Table 4 and relate to many of the variables discussed above. One of the major objectives of this literature review was to familiarize the research team with as much literature (i.e.

Table 3. Hydrocarbons in waters of southern California Bight in 1977 nanograms/liter (ppt) of dissolved phase from (deLappe et al. 1980). . ,

Location	Depth	Unresolved HC	Resolved HC	N-Alkanes
Coal Oil Point				
Winter	3- 5m	320	45	12+3
18-24 Feb	13- 33 m	360	45	21.0
	56 m	160	34	8.4+4.9
Summer	3- 5m	110	42	1.9
28 Aug-1 Sep	13- 33 m	79	15	2.5
	56 m	50	21	1.6
Santa Monica Bay				
Winter	3 - 5 m	610	110	17.0
18-24 Feb	37- 45 m	380	58	19.0
	874-885 m	550	71	18
Summer	3- 5m	73	38	1.7
28 Aug-1 Sep	37- 45 m	64	28	3.0
	874-885 m	150	13	3.2
Huntington Beach				
Winter	3- 5m	430	120	23.0
18-24 Feb	12- 17m	220	49	8.1
	20- 34m	740	300	27.0
Summer	3- 5m	180	80	5.7
28 Aug-1 Sep	12- 17 m	91	22	4.3
	20- 34 m	100	56	4.7
San Miguel Island				
Winter	3- 5m	290	63	13.0
18-24 Feb	10- 34 m	240	22	8.1
	196-237 m	160	68	2.1
Summer	3- 5m	99	110	6.9
28 Aug-1 Sep	10- 34 m	97	120	5.9
	---	--	--	--
Santa Rosa Island				
Winter	3- 5m	110	14	3.9
18-24 Feb	40- 50 m	390 "	100	23.0
	95-108 m	380 .	110	23.0
Summer	3- 5m	53	17	1.9
28 Aug-1 Sep	40- 50 m	110	36	3.6
	95-108 m	67	17	3.0

Table 3 (Cent).

Location	Depth	Unresolved HC	Resolved HC	N-Alkanes
Santa Cruz Basin				
Winter	3- 5m	150	36	4.6
18-24 Feb	19- 34 m	200	62	13.0
	1839-1857 m	330	97	11.0
Summer	3- 5m	69	34	4.2
28 Aug-1 Sep	19- 34 m	50	23	2.0
	1839-1857 m	53	35	2.7
San Nicolas Basin				
Winter	3- 5m	250	48	16.0
18-24 Feb	21- 34 m	470	110	63.0
	1720-1762 m	250	80	44.0
Summer	3- 5 m	87	34	2.6
28 Aug-1 Sep	21- 34 m	120	29	2.0
	1720-1762 m	97	12	1.5
Santa Barbara Basin				
Winter	3- 5m	280+77	74+26	15+8
18-24 Feb	17- 40 m	380±130	64T16	28±13
	558-564 m	360±88	70±1 0	15±5
Summer	3- 5 m	41	14	1.3
28 Aug-1 Sep	17- 40 m	145	1100	2.6
	558-564 m	140	24	2.9
San Pedro Basin				
Winter	3 - 5 m	180	15	9.3
18-24 Feb	435-440 m		15	4.5
	863-871 m	1;:	17	6.5
Summer	3- 5m	110	42	4.5
28 Aug-1 Sep	435-440 m	94	12	3.9
	863-871 m	96	12	4.5
Tanner Bank				
Winter	3- 5m	450	44	12.0
18-24 Feb	19- 32 m	330	35	12.0
	62- 73 m	260 "	72	16.0
Summer	3- 5m	140 .	29	2.9
28 Aug-1 Sep	19- 32 m	110	72	4.8
	62- 73 m	140	17	1.5

Table 4. Critical variables which must be considered for laboratory experimental results to be useful for environmental predictions.

1.	Use of experimental organisms occurring in the area of concern.
2*	Use of all life history stages of the experimental organism.
3.	Type of hydrocarbon tested (i.e. must be hydrocarbon likely to be encountered in the particular geographic area of concern).
4*	Hydrocarbon concentration (i.e. must be realistic in terms of environmental levels likely to be encountered).
5.	Duration of the experiments (i.e. must be realistic in terms of probable exposure time in nature).
6.	Experimental regime (i.e. should be flow-through to approximate natural conditions).
7.	Lethal as well as sublethal effects should be examined.

results and limitations of previous work} as possible related to laboratory experiments with oil and organisms. A second objective was to acquire LD50 (Table 1) values for use as a guide in choosing experimental concentrations to be administered in the BLM Commercial Fish and Shellfish Toxicity studies, since all other experimental conditions had been determined prior to the literature review. Of the studies reviewed in this report, none incorporated all of the critical experimental variables discussed above (Table 2), and few contained information relevant to the needs of the BLM related to commercial and sport fisheries along the California coast.

MOLLUSCA

GAMETE STUDIES

Few studies have addressed the effects of petroleum hydrocarbons (PHC) on molluscan gametes. In experiments which concentrated on the effects of PHC on gametes, observations generally were restricted to fertilization and developmental success after various permutations of exposure. Sublethal studies are practically non-existent. Renzoni

(1973) examined the effect of water soluble fractions (WSF) from fuel and crude oil on gametes and larvae of mussels and oysters (Mytilus galloprovincial is, Crassostrea angulata, C. gigas). Normal fertilization was observed for all species in all experimental regimes, however, complete development was inhibited in high WSF concentrations (100 and 1,000 ppm) of Venezuelan and Russian crude. In experiments where eggs (of Mulina lateralis and Crassostrea virginica) were exposed to WSF prior to mixing with untreated sperm, Renzoni (1975) showed that the degree of toxicity to fertilization was correlated to petroleum hydrocarbon concentration (Tables 5 and 6). Similar results were obtained when spermatozoa exposed to WSF were mixed with untreated eggs of M. lateral is.

Whipple et al. (1978) conducted experiments designed to examine effects of WSF Cook Inlet crude on spawning stages of the common littleneck clam (Protothaca staminea). After exposure to concentrations of 90-100 ppb for 4 days, clams were histologically examined for developmental stage of maturing eggs, presence of abnormal or dead eggs, and the presence of motile spermatozoa. Initial results showed that monocylic aromatics in whole clam tissue were bioaccumulated ($\bar{X}$=1.20 ppm [micrograms/gram wet weight]). Female clams had the highest levels, with the ripest female supporting tissue concentrations 28 times higher than those in the water regime. In animals autopsied during the exposure period, no mortality of eggs or spermatozoa was observed. However, after one week of deputation, dead eggs and spermatozoa were found in the gonads indicating a lag period existed between exposure and the time effects were manifested in the organism. Similar experiments (Whipple et al. 1978) with the Japanese littleneck clam (Tapes semidecussata) preliminarily indicated that the common littleneck was more sensitive to . petroleum hydrocarbons.

Sublethal developmental abnormalities noted by Renzoni (1975) in embryos exposed to WSF of Nigerian and Alaskan crude oil included enlarged embryos, variable embryo shape, and straight hinge stages which lacked the veliger shell.

Table 5. Crassostrea virginica: percentages of fertilization, of embryo development and larval survival at various oil-in-water concentrations*., All values are averaged for duplicate cultures and are expressed as percentage of the values obtained from control cultures.

Crude Oil Origin	Concn of the Oil (ml/l-l	% of Fertilization	% Development of Embryos	% Survival of Larvae
	0.001	89.9	91.5	83.0
Alaska	0.01	88.2	86.5	80.2
(Prudhoe)	0.1	83.7	81.9	75.2
	1.0	71.4	68.5	55.0
	0.001	90.1	89.0	77.1
Nigerian	0.01	80.3	77.9	72.5
(Bonny)	0.1	75.2	66.4	68.7
	1.0	63.3	50.4	43.3
	0.001	89.9	92.8	88.3
Kuwait	0.01	88.7	88.9	81.8
	0.1	85.2	85.9	77.7
	1.0	73.2	69.3	53.8

Table 6. Mulinia lateralis: percentages of fertilization, of embryo development, of larval survival and of the increase in mean length at various oil-in-water concentrations*. All values are averaged for duplicate cultures and are expressed as percentage of the values obtained from control cultures.

Crude Oil Origin	Concn of the Oil (ml/l-l	% of Fertilization	% Development of Embryos	% Survival of Larvae	% Increase in Mean Length of Larvae
	0.001	93.8	91.9	84.8	82.8
Alaska	0.01	91.9	89.0	85.3	82.5
(Prudhoe)	0.1	85.1	86.9	80.6	74.5
	1.0	68.8	77.1	71.0	55.6
	0.001	89.4	89.0	86.4	81.7
Nigeria	0.01	88.5	87.0	82.6	72.6
(Bonny)	0.1	83.7	77.8	71.3	63.3
	1.0	67.8	62.8	59.2	40.3
	0.001	96.8	95.8	88.0	81.3
Kuwait	0.01	94.2	94.1	83.8	82.5
	0.1	89.5	91.1	80.9	74.5
	1.0	73.8	72.9	62.0	50.1

*from Renzoni 1975

Finally (Renzoni 1973, 1975) noted that spermatozoa were particularly sensitive to WSF in water or in combination with dispersants and water. Effects were manifested in reduced fertilization capability.

LARVAL STUDIES

Lethal

Larval life stages of marine organisms represent a vulnerable critical period in the developmental life history. Studies of molluscan larval forms and their response to petroleum hydrocarbon exposure have been limited and have focused on developmental success.

Renzoni (1973) exposed fertilized oyster and mussel eggs (Crassostrea angulata, C. gigas, Mytilus galloprovincial is) to WSF of Venezuelan crude and No. 1 fuel oil. Successful larval development was assayed by noting the number of swimming larvae. Significant reduction in developmental success was recorded in the highest (1,000 ppm) concentration (Table 7). Larvae developing in lower (more realistic with regards to environmental levels) concentrations of oil in water displayed success equal to that of controls.

Table 7. Average number (%) of larvae developed in water with oil B and with derivative E*.

Species	Control	1 ppm		10 ppm		100 ppm		1000 ppm	
		B	E	B	E	B	E	B	E
C. angulata	84.6	84.3	83.6	83.3	83.6	83.0	81.3	69.3	74.0
C. gigas	84.3	83.6	82.3	81.6	83.3	79.3	83.6	68.6	73.0
M. gallo-provincialis	87.3	84.0	83.3	82.3	85.6	81.0	84.3	71.6	75.6

*from Renzoni 1973

Sublethal

In experiments where fertilization was allowed to take place in an oiled water environment, Renzoni (1973) observed "first movement activity." Movement of larvae maintained in lower concentrations (1 and 10 ppm) was similar to controls, but those exposed to higher concentrations (100 and 1,00 ppm) of crude and fuel oil displayed reduced swimming speed, irregular swimming, and total inactivity.

Experiments with WSF of Nigerian and Prudhoe Bay crude oils on bivalve larvae (Renzoni 1975) produced survival and growth data (Tables 5 and 6). In Mulinia lateralis and Crassostrea virginica growth of larvae was significantly inhibited by high oil in water concentrations (1 and 0.1 milliliter/liter). Behavioral abnormalities appearing in larvae exposed to all concentration levels included irregular swimming, and some larvae swimming with velure constantly protruding from the shell bearing cilia that alternated periods of movement with periods of inactivity. It was also noted that all effects were more exaggerated in animals exposed to Nigerian versus Alaskan crude oil.

ADULT STUDIES

Lethal

Toxicity studies with mollusks have been confined primarily to clams, mussels, and oysters, many of which are of commercial or sportfish importance in the United States, but not necessarily in California. Considerable variation exists between studies with regards to organism exposed, hydrocarbon tested, concentration administered, duration of exposure, and system of exposure (i.e. static versus flow-through). Acute toxicity tests including LD50s (as well as others for adult life stages) were rare among the molluscan studies (Table 1). Since one or more of these, variables differs between all of the studies, comparisons should be made cautiously.

Aqueous extracts of crude oil commonly contain the water soluble (WSF) aromatic hydrocarbons (McAuliffe 1966). Short-term experiments conducted by Nunes and Benville (1978) administering WSF of Cook Inlet crude to Manilla clams (Tapes semidecussata) in both static and flow-through systems produced different results. Animals (from "pristine" environments) exposed to 7.35 ppm total hydrocarbons for 96 hours in static systems reached LD50 levels by hour 72 (Table 2). However, animals collected from "polluted" areas and tested under identical conditions sustained only 30% mortality in 96 hours. Manilla clams exposed to the same WSF in slightly lower concentrations ($\bar{X}$=3.16 ppm) for 8 days in a flow-through system sustained only a 40% mortality. These disparate results suggest that the type of exposure system can significantly affect test results. Further, any extrapolations or environmental predictions based on results of static bioassays should be suspect.

Accumulation and release of WSF aromatics from No. 2 fuel oil was examined by Neff et al. (1976) in the clam, Rangia cuneata, and the oyster, Crassostrea virginica. In static experimental regimes for 4 days with WSF oil concentrations of approximately 6.8 ppm, oysters bioaccumulated 96.7 ppm PHC in their tissues. When allowed to depurate these levels dropped. The hydrocarbons identified in the tissues included n-paraffins and aromatic hydrocarbons in the boiling point range from naphthalene to dimethyl -phenanthrene. In a flow-through system with exposure lasting 8 hours, hydrocarbon levels in oyster tissues were high (311.7 ppm) and n-paraffins dominated the contaminant list. When allowed to depurate for 24 hours their tissues still contained considerably amounts of hydrocarbons (66.7 ppm). Clams exposed to the same conditions reacted similarly but accumulated much smaller amounts of the hydrocarbons (89.6 ppm). They too reduced tissue levels when allowed to depurate. Continued observations, for latent effects either lethal or sublethal were not performed. However, tissue levels were monitored for up to 672 hours following exposure and PHC concentrations dropped to 0.1 ppm. Generally in these experiments with clams and oysters naphthalenes appeared to be the hydrocarbon bioaccumulated the most and retained the longest.

Relative toxicity of No. 2 and No. 5 fuel oils in short-term exposure experiments on the mussel, Mytilus edulis, was investigated by Clark and Finley (1975). After exposure to surface slicks of the oils for 48 and 32 hours, mortalities of 45% and 35%, respectively, for No. 2 and No. 5 oils were recorded. Surviving animals maintained in clean seawater for up to 35 days after the experiments still contained traces of fuel oil residues in their tissues. However, no sublethal health evaluations of the surviving animals were performed.

Lee, Sauerheber, and Benson (1972) exposed M. edulis to several individual hydrocarbon compounds including mineral oil, [^{14}C] hapta-decane, 1,2,3,4 tetrahydronaphthalene, [^{14}C] toluene, [^{14}C] naphthalene, [^{3}H] 3,4-benzopyrene for periods of 4, 6, and 76 hours. Though exposure concentrations varied, the non-toxic paraffinic hydrocarbons were generally taken up to a much greater extent (10 milligrams per mussel) than the aromatic hydrocarbons (2 to 20 micrograms per mussel). After exposure, animals placed in clean seawater discharged most of the hydrocarbons. However, the sublethal effects of the retained compounds (or latent effects of exposure) on surviving individuals was not investigated further.

Long-term studies (>96 hours) comprise the majority of literature dealing with effects of hydrocarbons on mollusks. As described for egg and larval studies, the test organisms were mussels, clams, and oysters. Experimental regimes and evaluation criteria varied between all studies.

Mussels have been used in several hydrocarbon studies because they are: 1) a hardy experimental test organism (Gil fillan 1973); 2) widely distributed and therefore available (Soot-Ryen 1955); 3) have been studied extensively and considerable physiological baseline data are available (Field 1922); 4) of a convenient size, small enough to be handled easily in the laboratory and large enough for specific organ dissection (Lee et al. 1972); 5) an important member of the intertidal ecosystem (Ricketts and Calvin 1968).

Experiments by Kanter et al. (1971) examined the tolerance of mussels (Mytilus californianus) from "polluted" and "non-polluted" areas to high dosages (1×10^3 and 1×10^4 ppm) of Santa Barbara crude oil. Mortality was higher and occurred sooner in animals that had not been previously exposed to oil. These studies were performed in static systems for 10-day periods, but the results compare favorably with the "tolerance" recorded by Nunes and Benville (1978) in later research on the Manilla clam.

Additional mortality studies on M. californianus in static systems performed by Kanter (1974) with Santa Barbara crude spanned greater time periods. Each set of experiments lasted approximately 60 days and experimental concentrations were 1×10^3, 1×10^4, and 1×10^5 ppm total crude in water. Even with these high oil dosages, mortality in experimental aquaria was limited to the highest concentrations. Kanter found that mortality differed between the populations tested (i.e. those animals collected from "clean" environments were more susceptible) and was dependent on size of the individuals and season. These experiments more closely approach the experimental regime desired for predictions on the California coast. However, unrealistically high oil concentrations and the use of a static system limit the utility of these results.

In animals exposed in the environment to natural oil seepage, deLappe et al. (1979) reported bioaccumulation of PHC in M. californianus above background levels. These results suggest that significant depuration does not take place even when exposure to the pollutant is intermittent.

Waste motor oil was tested by Gardner et al. (1975) on the oyster, Crassostrea virginica, and scallop, Aquipectins irradians. Both lethal and sublethal observations were made on organisms after exposure to concentrations of 0, 20, 100, and 250 ppm. Mortality in oysters after a 60-day exposure was 32% and 68%, respectively, for concentrations of 20 and 100 ppm. Scallops exposed to 20 ppm for 21 days suffered 60%

mortality. Although acute LD50 levels were not determined for these species, sublethal effects (discussed in following sections) were detected under the differing exposure regimes.

Sublethal

Sublethal effects following long-term exposure studies were examined by several investigators. Experimental data included records of mortality along with sublethal effects. Sublethal observations were made on bioaccumulation, deputation capability, and evidence of physiological and histological impairment.

The ability of bivalves to degrade petroleum aromatic hydrocarbons after acute exposure was investigated by Vandermeulen and Penrose (1978). After exposing Mya arenaria, Mytilus edulis, and Ostrea edulis to WSF of Kuwait crude or Bunker C (fuel oil) none of the animals showed any evidence of hydrocarbon-induced aryl hydrocarbon hydroxylase or N-demethylase activity. This inability to produce petroleum degrading compounds, and the tendency for PHC to bioaccumulate in tissues suggests that PHC compounds could be transferred, unaltered into the food chain.

Crassostrea virginica exposed to No. 2 fuel oil for 50 days and allowed to depurate selectively, retained aromatic hydrocarbons (Stegeman and Teal 1973). The retained PHC were found concentrated in the lipids of body fat. In addition, aliphatic fractions of the hydrocarbons retained in the oyster showed a degraded appearance, possibly indicating that oysters themselves are modifying the oil through internal enzyme systems. This hypothesized enzyme activity was not found in other bivalves (Vandermeulen and Penrose 1978).

Selective retention of aromatic hydrocarbons in body tissues was also found by Fong (1976). In 10-day experiments, exposing the soft shell clam Mya arenaria to WSF of Kuwait crude (90 to 380 ppb), aromatic hydrocarbons were incorporated into the internal organs particularly

those high in lipid content, e.g. the intestine and hepatopancreas. In a second set of experiments, Fong found oxygen consumption was elevated in clams exposed to 220 to 370 ppb oil for 21 days.

Bioaccumulation of aromatic hydrocarbons from Cook Inlet crude was recorded in the Manilla clam Tapes semidecussata (Nunes and Benville 1979). After 8 days of exposure in a flow-through system to 3.1 ppm, total hydrocarbon levels in tissues were high for benzene, toluene, m-xylene, o-xylene, and ethylbenzene. After a week of deputation, tissue levels had decreased slightly but thereafter remained relatively constant.

Measurement of sublethal effects resulting from exposure to hydrocarbons can take many forms. Physiological, behavioral, or histological observations are among the most common techniques employed. By comparison with control results, observations on experimental animals can be assessed in terms of deviations from the norm.

Byssus thread production by mytil ids is a common, daily requirement for this species in nature. Byssus thread production was found to be affected by the presence of hydrocarbons in surrounding waters. Enhancement and/or depression of byssus thread production was recorded by Carr and Reish (1978) in mussels (M. edulis) exposed for periods between 1 and 14 days to extracts of crude oil, No. 2 fuel oil, and outboard motor oil. Although the exposure system apparently was static, concentrations of toxicant administered was not accurately assessed.

Carbon flux as measured by respiration was depressed in mussels (M. edulis) exposed to WSF of crude oil (Gilfillan 1975). Physiological stress was also noted by Fossato and Canzonier (1976) in M. edulis exposed to diesel fuel for 15 to 20 days.

Histological observations made on oysters, Crassostrea virginica after a 36-day exposure to 100 ppm oil, revealed several abnormalities

(Gardner et al. 1975). Localized clots and dark pigmented areas in cardiac musculature were evident. Lesions were obvious in branchial vasculature and other clots apparently occluding circulation were widespread. In addition, deposition of granulation in the gastrointestinal tract was also noted. In scallops (Aequipectin irradians) exposed to waste oil at 100 and 500 ppm for 24 and 6 hours, branchial and renal lesions were present (Gardner et al. 1975). Lesions were not recorded in these tissues after 21 days of exposure to 20 ppm oil. Branchial, ciliated columnar epitheliums was either necrotic or lacking following exposure to 500 ppm of waste oil; these cells further lacked their compliment of cilia after exposure to 100 ppm of oil. Although these observations were significant, no health or ecological implications of such findings were discussed.

Stainken (1978) found altered and modified respiratory rates in the clam Mya arenaria following exposure to No. 2 fuel oil. Oxygen consumption generally rose as stress from elevated hydrocarbon levels increased. In addition, responsiveness to tactile stimulation was depressed and almost non-existent when hydrocarbon levels were high (e.g. 100 ppm). When allowed to depurate, oxygen consumption correspondingly decreased; however, some hydrocarbons remained in tissues even after deputation. Again the ecological implications of these findings and how they apply to the organisms survival in the natural environment were not addressed.

Keck et al. (1978) found growth in the hard clam, Mercenaria mercenaria, exposed to Nigerian crude was severely reduced compared to control organisms. Further, this reduction in growth was directly related to the concentration of toxicant administered. In addition, the authors' cited latent effects not recorded during the six-week experimental period. Latent effects, primarily death; were found to occur during the two-week deputation period which followed the experiments.

CRUSTACEA

EGG STUDIES

Little is known of the effects of petroleum hydrocarbons on the eggs of marine crustaceans. The existence of eggs is largely a short-term phenomenon among marine crustaceans, as many species have relatively short hatching times. Compounding the difficulties in determining effects only on eggs is the behavior of many groups which tend eggs, or, as in the amphipods and isopods, hold the eggs in a brood pouch until hatching.

Under these conditions the effects of petroleum hydrocarbons on eggs have largely been documented only in terms of the creation of the eggs. Reproductive behavior in the shore crab, Pachygrapsus crassipes, was eliminated by exposures to soluble crude oil in quantities possibly below 10 ppb (Takahashi and Kittredge 1973). Abnormal reproductive behavior has been reported in the fiddler crab, Uca pugnax, following pollution by No. 2 fuel oil. Straughan (1971) found barnacles' breeding efforts were affected in the low intertidal area, but not in the high intertidal area.

Tatem (1976) exposed gravid female grass shrimp (Palaemonetes pugio) to the water soluble components of No. 2 fuel oil. No larvae hatched during the exposure period. After exposure for 72 hours, control females produced an average of 45 larvae, females exposed to 0.24 ppm petroleum hydrocarbons produced an average of 48 larvae, those exposed to 0.72 ppm averaged 35, and those exposed to 1.44 ppm averaged only 9 healthy larvae.

Linden (1976a,b) reported the amphipod Gammarus oceanicus entered the precopulation stage less frequently when exposed to oil, and that gravid females exposed to sublethal quantities of water soluble crude oil greatly decreased their brood numbers. Whether this was a direct effect upon the eggs (i.e. the oil was toxic to the eggs themselves) or an

indirect effect (i.e. the oil affected the female who then did not produce or perhaps resorbed the eggs) is a moot point, but indicates the difficulties in attempting to determine petroleum hydrocarbon effect on crustacean eggs.

-LARVAE STUDIES

Lethal Effects

Wells and Sprague (1976) found first stage larvae of the American lobster (Homarus americanus) most sensitive to the water soluble fraction (WSF) of crude oil in 96 hour LC50 testing, with third and fourth stage larvae testing at values of oil five times as great (Table 1). Brodersen et al . (1977) measured LC50 values for 1 arvae of king crab (Paralithodes camtschatica) , kelp shrimp (Eualus suckleyi), and all six larval stages of the coonstripe shrimp (Pandalus hypsinotus). Consideration of different criteria for death varied the LC50 values calculated (Table 1).

Caldwell et al. (1977) tested the toxicity of the WSF and its major aromatic components on the larval stages of the dungeness crab Cancer magister. The lowest concentration at which toxic effects were seen was 0.22 ppm, with the naphthalene concentration placed at 0.0049 ppm. Acute toxicity of the 12 compounds tested was related to the degree of alkyl substitution. Naphthalene and its derivatives were more toxic than benzene and its derivatives. In long-term exposures benzene contributed a greater fraction of the WSF toxicity, however, and the authors suggest this toxicity may be the result of a different mechanism than that of acute tests.

Anderson (1979) in his comprehensive survey concluded larval crustaceans were more susceptible to petroleum hydrocarbon (PHC) toxicity, but sensitivity was two or three times that of the adults, and not of orders of magnitude.

Sublethal Effects

Measures of sublethal effects of petroleum hydrocarbons on crustacean larvae have been accomplished largely in the areas of effects on growth, molting, and movement. Linden (1976a,b) found PHC exposure decreased growth rate in the larvae of the amphipod Gammarus oceanicus. Cox and Anderson (1973) found growth of young brown shrimp (Panaeus aztecus) was unaffected by a limited exposure to the WSF of No. 2 fuel oil, although Tatem (1976) found the same toxicant significantly reduced the growth rate of grass shrimp (Palaemonetes pugio). Johns and Pechenik (1980) reported reduced growth in larvae of the crab Cancer irroratus upon exposure to the water-accommodated fraction of No. 2 fuel oil, and also noted the metabolic cost of body maintenance increased. Milovidova (1974) reported reduced growth rates in larvae of the Black Sea isopod Idotea baltica basteri at 10 ppm WSF of crude oil, but no reduction at 1.0 ppm. Working with an Arctic isopod Saduria entomon, Percy (1978) found little effect of crude oil WSF on growth and molting, and concluded these parameters were not good measures of petroleum hydrocarbon effects in such animals. Consideration of what a larva truly is may be of some importance, as many crustaceans have brood larval forms, different from the adults, while isopods and amphipods are released from the brood pouches nearly in the form of miniature adults.

Somewhat related to the effects on growth are the effects on molting, a necessary adjunct to growth. Several studies (Wells 1972; Wells and Sprague 1976, on lobsters; Rice et al. 1976 on pandalid shrimp; Karinen and Rice 1974 on a decapod; and others) have established or strongly indicated greatest mortality occurs during molting. Crustaceans are naturally most vulnerable in the hours immediately following molting and PHC exposure has its greatest impact at these times. Brodersen et al. (1977) have pointed out that this should be kept in mind when comparing studies, as the impact of PHC exposure may vary depending on whether a molting occurs during exposure as opposed to exposure strictly during an intermolt phase. Such differences may also partially account for the differing results of PHC exposure tests.

A third parameter examined for larval PHC exposure is movement. Ferns (1977) reported planktonic larval lobsters exposed to 0.1 ppm WSF of crude oil in a flow-through system remained aggressive, active, and locomotor, while larvae exposed to 1.0 ppm lacked this movement. Donahue et al. (1977) noted lessened activity in barnacle (Chthamalus and Balanus) larvae exposed to the WSF of No. 2 fuel oil. Corner et al. (1968) found 50% inactivation of Stage II nauplii of the barnacle Elminius modestus after eight minutes exposure to 10 ppm kerosene extract. Crisp et al. (1967) noted this narcosis in testing with the same species, and found that substitution of polar groups into paraffin structure or increased molecular symmetry increased the narcotic effect, listing toxicants effects as: phenol < benzene < cyclohexane < heptane. Sanborn and Malins (1977) reported such narcotization in spot shrimp (Pandalus platyceros) larvae and dungeness crab (Cancer magister) zoeae exposed to 8 to 12 ppb naphthalene under continuous flow conditions. Rice et al. (1976) found 96 hour doses of crude oil WSF caused several crustacean species' larvae to be incapable of locomotion, with 50% immobilization occurring in shrimp larvae (Pandalus hypsinotus and Eualus fabricii) at 0.75 ppm and Tanner crab larvae (Chionoecetes baridi) at 1.20 ppm. Dungeness crab larvae suffered immobilization at 1.7 ppm. This lack of mobility may have critical effects on other functions. Tatem (1976) reported the larval grass shrimp suffering slowed growth upon exposure to the WSF of No. 2 fuel oil were not moving to food, being much less active than larvae in the control group.

Bigford (1977) tested effects of the water-accommodated-fraction of No. 2 fuel oil on the larval stages of the rock crab, Cancer irroratus, in terms of other parameters. In general geonegative movements were depressed in early-stage larvae, and enhanced in later stage larvae. Phototactic behaviors were significantly changed, and pressure responses were slightly affected. Exposures at 1.0 ppm and 0.1 ppm concentrations were performed.

ADULT STUDIES

Atema and Stein (1974) found a water-soluble fraction (WSF) concentration of LaRosa crude oil of 17 ppb doubled the response time for the American lobster, Homarus americanus, to notice and to pursue food. Milovidova (1974) exposed the amphipod Gammarus olivii to emulsions of crude oil, and observed reduced feeding rates with increasing amounts of oil. However, Crapp (1971) concluded that the feeding rate of the common shore crab, Carcinas maenas, remained unaffected over 12 weeks of exposure to crude oil WSF. Takahashi and Kittredge (1973) found exposures to "1 0W" concentrations (they did not determine exact quantities) of the WSF of a Sisquoc crude oil and a California crude oil from the lower miocene totally inhibited feeding response to soluble food in the American Lobster. Blumer et al. (1973) noted that the chemoreceptor-mediated behavior of lobsters was disrupted after exposure to oil in the water column, but electron micrographs showed no histological damage. Kittredge et al. (1974) suggested that oil exposure destroyed the dendritic tip in the aesthetascs.

Takahashi and Kittredge (1973) also found the shore crab Pachygrapsus crassipes responded to various amino acids, but this response was eliminated by 24 hour exposure to the WSF of crude oil, estimated at 10 ppb. Inhibition by monoaromatic hydrocarbons was brief, with recovery in 30 to 60 minutes. Polynuclear aromatics delayed recovery of the feeding response, 1 ppb naphthalene delayed recovery for 3 or more days. The crude oil WSF caused a delay of sensitivity of 3 to 6 days after the animals were transferred to clean water.

Busdosh (in press) examined these effects in the amphipod Boeckosimus (=Onisimus) affinis. Animals constantly exposed to six solutions with a range of 5.2 to 0.019 ppm experienced a reduced food search success relative to the strength of the WSF. After two weeks of exposure the feeding success rates gradually increased. Animals exposed for 3 or 10 days and then removed to clean water also showed decreased food search

success related to the strength of the WSF and the duration of exposure, but a general recovery to near the success rate of control animals occurred after two weeks in clean seawater. Percy (1976) noted this same amphipod showed a distinct preference for unoiled over oiled food, although the isopod Saduria (=Mesidotea) entomon exhibited no discrimination. Busdosh et al. (1978) reported a reduction in food search success in B. affinis for the first month of exposure to oiled sediment, with a recovery to near control animals' rates after that. This may indicate the lighter weight, soluble components of the oil caused the inhibition, and affected the animals as a WSF after leaching from the sediment. All oiled sediment experiments must be examined in light of the possibility the effects may be originating from oil dissolved in a thin layer above the substrate, or in interstitial waters.

Pearson and Olla (1977) demonstrated the blue crab's (Callinectes sapidus) response to food can be noted by antennular flicking, and in a later work (Pearson and Olla 1979) reported the crab could detect naphthalene at 120 ppb, an indication of the sensitivity of the chemoreception apparatus being affected by oil exposures.

Blackman (1972) found the shrimp Crangon crangon was likely to ingest Kuwait crude oil, and contact with the oil reduced the overall feeding rate. The oil adhered to the foregut, with some remaining until the next molt. This may allow oil to pass on to predators, especially if the presence of the oil affects behavior or the specific gravity of the shrimp.

Respiration has long been a standard parameter in physiology studies, and was a logical inclusion when sublethal effects of PHCS came under examination. Johnson (1977) has completed an excellent review of respiration studies on oil-treated crustaceans, and the following is from his review:

> "The respiration rates of various crustaceans are sensitive to the presence of petroleum, but the direction or

magnitude of respiratory change that may be produced by a given dose of petroleum is unpredictable. Anderson et al. (1974) showed that the oxygen consumption rate of the mysid Mysidopsis almyra increases with exposure to water-soluble fractions of No. 2 fuel oil (0.10 to 0.58 ppm total naphthalenes) and to the oil-water dispersion of No. 2 fuel oil (1 to 10 ppm total hydrocarbons). The maximum respiratory rate enhancement for M. almyra occurred at 0.4 ppm total naphthalenes for both water-soluble fraction and oil-water dispersion exposures.

"Grass shrimp (Palaemonetes pugio) exposed to 3.0 to 3.6 ppm of the oil-water dispersion of No. 2 fuel oil (containing 0.10 ppm total naphthalenes) for five hours showed a decrease in content of 2.0 ppm (Anderson 1975, Tatem 1976). After the oil-exposed grass shrimp were allowed to depurate in clean seawater for seven days, the tissue naphthalene content had decreased to background levels and the respiration rate had returned to normal. The respiration rate of postlarval brown shrimp, Penaeus aztecus, also tended to decrease during exposure to low concentrations of the WSF of No. 2 fuel oil or South Louisiana crude oil. At higher concentrations, the brown shrimp developed an increased respiration rate accompanied by increased locomotor activity and abnormal swimming behavior. These effects were more pronounced with larger animals. The responses exhibited by the brown shrimp were also greater when the animals were exposed to the WSF of No. 2 fuel oil than when exposed to the WSF of South Louisiana crude oil. The authors noted that levels of hydrocarbons in the tissues and the size (or stage of development) of the shrimp may be factors influencing their respiratory response to petroleum hydrocarbons. Steed and Copeland (1967) found that petrochemical waste, at levels below that required to produce 50% mortality in 48 hours, depressed the respiration rate of P. aztecus, but increased the respiration of P. duorarum (pink shrimp).

"Rice et al . (1976) studied the effects of the WSF of Cook Inlet crude oil on the respiration of Alaska king crab - . (Paralithodes camtschatica). They found that a 5 hour exposure of the juvenile crabs to sublethal levels (<6 ppm) of the WSF had little effect on respiration, but exposure to levels which would kill the animals within 96 hours (>6 ppm) caused a prompt depression in oxygen consumption. The shore crab Carcinus maenas developed a variable pattern of respiratory responses when exposed to a seawater extract of Mid-Continent (United States) sweet crude oil (Yentsch et al. 1973). A mixture containing 1% of the extract in seawater (about 0.125 ppm total hydrocarbons) caused a slight reduction (about 20%) in oxygen consumption of the crabs; however, a mixture containing 10% extract (1.25 ppm total hydrocarbons) caused an increase in the respiration rate by about 50%.

"Percy and Mullin (1975) reported that the Arctic marine amphipod Onisimus affinis also exhibited variable respiratory responses when dplaced in dispersions of crude oil in sea-water. Four crude oils were tested: Atkinson Point, Venezuela, Pembina, and Norman Wells. Oxygen consumption of the amphipods was invariably depressed (by 7 to 22%) at the lower concentrations of all oils tested. This effect was reversed at higher concentrations of crude oil. 'Heavy' dispersions of all the crude oils with the exception of Atkinson Point crude oil stimulated respiratory rates of the amphipods by as much as 40% over control rates. The oils which produced the greatest respiratory depression at low concentrations generally produced the least stimulation at high concentrations. Respiration of cell-free homogenates, however, was enhanced 10 to 45% by exposure to seawater dispersions of Normal Wells crude oil at all concentrations tested."

An additional study performed by Edwards (1978) found the shrimp Crangon crangon exposed to 1 to 2 ppm of the WSF of crude oil showed respiration not significantly differing from those of control animals. A significant decline in respiration rate was manifested in animals exposed to concentrations of 3 to 6 ppm. Busdosh and Atlas (1976) fractionated crude oil to paraffinic, aromatic, and asphaltic fractions, and found significant reduction in the respiration rates of the amphipod Boeckosimus affinis exposed to the WSF of the paraffinic and aromatic fractions, but not in the asphaltic fraction. A significant decrease was also experienced in the presence of the WSF of diesel. Bakke and Skjoldal (1979) found no respiration effects caused by the WSF of toluene in the isopod Cirolana borealis although "behavioral disturbances" did occur.

The varying and seemingly conflicting effects of the WSF on respiration may originate in differing effects on basal and active metabolisms, or a combination. Percy (1977) has eloquently discussed these ideas, theorizing these possible varying mechanisms' effects, and pointing out the present shortcomings of respiration testing.

Movement has been shown to be affected by the WSF, usually by reduction. Swedmark et al. (1973) found locomotion in the prawn Leander adspersus ceased at all concentrations (<350 ppm) of Oman crude oil dispersions tested. Impaired activity was also noted for the spider crab, Hyas araneus, and hermit crab, Eupagurus bernhardus exposed to diesel emulsions, but partial recovery was achieved following deputation in clean water. Mironov (1970) determined that 10 ppm of crude oil in the water column caused hermit crabs to become sluggish. Rice et al. (1976) found small doses of the WSF of crude oil were sufficient to render larvae of the shrimps Pandalus hypsinotus and Eualus fabricii; the Tanner crab, Chionoecetes bairdi; and the dungeness crab, Cancer magister, incapable of normal locomotion. Tatem (1977) found larvae of the grass shrimp, Palaemonetes pugio, when exposed continuously to the WSF of No. 2 crude oil (0.55 to 0.85 ppm) were not nearly as active as control larvae. Adults exposed to the same pollutant, however, showed increased activity

when compared to control animals. An increase was also noted by Bean et al . (1973) who reported the pandal id shrimp Pandal us danae di spl ayed "agitated" and possibly "searching" behavior when exposed to a 1 to 2 ppm extract of water soluble oil, while control animals rested.

Percy and Mullin (1977) found even low WSF concentrations of crude oil significantly impaired locomotor activity in the amphipod Boeckosimus (=Onisimus) affinis. Percy (1977) reported this species moved away from sediment that was oil contaminated, but noted a second amphipod species Corophium clarencense) and two congeneric isopods, Saduria (= Mesidotea) entomon and S. sibirica, did not move away. Busdosh et al. (1978) also reported B. affinis preferred clean sediment over oiled sediment for the first month of exposure, after which the discrimination lessened. Boeckosimus affinis also showed reduction in distance moved and percentage of time spent moving while exposed to the WSF of crude oil (solution from 5.2 to 0.019 ppm). Animals exposed for 3 to 10 days then removed to clean water showed a reduction of movement relative to the strength of the WSF and duration of exposure, with recovery of movement in a week or two (Busdosh in press). Although the distance moved by animals one-time exposed to the WSF was significantly lower than that shown by control animals, the amount of time spent moving was not significantly affected, in contrast to those animals constantly exposed to the WSF.

Linden (1976a,b) reported impaired swimming performance in the amhipod Gammarus oceanicus upon exposure to oil-water-dispersion of crude oil and No. 1 and No. 4 fuel oils.

Krebs and Burns (1977), working from oiled sediments from the Buzzards Bay spill, reported the high oil content of the sediment reduced population densities, male-female ratios, and juvenile settlement, and caused heavy overwinter mortality, locomotor reduction, and burrowing impairment in the fiddler crab, Uca pugnax.

While the WSF of crude oils and refined products does affect locomotion in marine benthos, both impaired and increased activity have

been recorded. Percy (1977), dealing with similar seemingly opposite effects in respiration at different petroleum hydrocarbon levels, has suggested different mechanisms may occur, affecting basal or active metabolism. Whatever the direction manifested, motion can be affected by the WSF of petroleum hydrocarbons.

Malins (1977), Varanasi and Malins (1977), Teal (1977), and Lee (1977) summarized the literature on food chain transfer, bioaccumulation, and turnover of hydrocarbons by marine organisms. These reviews have emphasized the biomagnification of hydrocarbons through the food web, as is typical for many pollutants. Most hydrocarbon accumulation, however, was directly from the water, with tissue levels containing 200 to 300 times the level of oil present in the surrounding water column.

Hodgins et al. (1977) have reviewed effects of petroleum hydrocarbons on disease and disease resistance in organisms, and have theorized that the presence of oil may increase the incidence of tumors as well as increased bacterial and other infections through suppression of immune responses.

Minichev and Brown (1979) found eye lesions in the brown shrimp, Penaeus aztecus, and suggested their link to sediment-bound residual crude oil. Neff et al. (1976) reported accumulation of petroleum hydrocarbons from No. fuel oil in juvenile brown shrimp. The animals initially quickly depurated hydrocarbons when moved to clean water, with a following slower release rate, to background levels after 250 hours. Cox and Anderson's (1973) study showed brown shrimp experienced maximum uptake of hydrocarbons from No. 2 fuel oil during the first hour of exposure. Although they continued to take in hydrocarbons, deputation also was initiated. Neither molting frequency or growth rates were affected. Anderson et al. (1974a) found brown shrimp accumulated an aromatic petroleum hydrocarbon to levels 10 times that of the water. Anderson et al. (1974b) reported a gradual release of aromatics when the animals were placed in clean water. Cox et al. (1975), working withthe

white shrimp, P. setiferus, showed larval forms had a peak of hydrocarbon concentration 48 hours after exposure to No. 2 fuel oil (38% aromatics)*, coinciding with the highest levels of aromatics in the water column. Neff et al. (1976) noted shrimp and fish are capable of metabolizing aromatic hydrocarbons, while bivalves seemingly cannot. In a study on oil tastes, Kneiper and Culley (1975) listed brown and white shrimps having an oil taste when exposed to an oil threshold level of 49 to 160 ppm of Louisiana crude oil. This taste was still present after animals were moved to clean water for one week.

Using radiolabeled hydrocarbons, Lee et al. (1976) found the blue crab eliminated most foreign hydrocarbons in the feces. All hydrocarbons were metabolized, with 50% of the radioactivity showing in the hepatopancreas after 25 days, and with no detectable evidence of radiolabeled material in any other tissues. Burns (1976) however, reported the fiddler crab, Unc pugnax could metabolize foreign hydrocarbons, but not rapidly enough to be of real use to the animal. Kneiper and Culley (1975) reported threshold levels of 620 to 1250 ppm for the presence of an oily taste in blue crabs.

Rohrbacher-Carls (1978) reported the amphipod Pontoporeia femorata concentrated two- and three-ring aromatic compounds from oiled sediment, with animals containing concentrations five times greater than that of the sediments. She also noted clearance of the PHCS with time. Rossi et al. (1978) examined PHCS i n the sand crab Emerita anal oga from southern California beaches, finding 0.9 to 24.1 *g/g dry weight saturated hydrocarbons, and 3.6 to 21.4 *g/g dry weight unsaturated hydrocarbons. Major constituents were n-alkanes 13.1% (in wt. % total), heneicosapentane plus heneicosahexane 1.0%, squalene 5.1%, and unidentified polyenes 7.8%. . Animals from an offshore island contained only biogenic compounds.

FISHES

EGG STUDIES

Lethal Effects

Anderson et al. (1977) investigated the effects of southern Louisiana crude (WSF) on embryos of Fundulus heteroclitus, and F. similus and found that hatching success decreased with exposure to increasing concentrations of the WSF. At 50% WSF (ea. 10 ppm total hydrocarbons) hatching success ranged from 40 to 72%. When the chorion of exposed eggs were experimentally removed, the mortality rate of embryos exposed to the 50% WSF increased.

Sharp et al (1979) exposed fertilized eggs of Fundulus heteroclitus to the WSF of No. 2 fuel oil for periods up to 35 days, and observed that hatching success was significantly depressed at 75% WSF (0.6 ppm total napthalenes). At concentrations <10% WSF hatching success was not depressed compared with controls. Larvae newly hatched from eggs exposed to 20% and 25% WSF for 35 days exhibited 61% and 0% survivorship, respectively. Ernst et al. (1977) exposed developing eggs of Fundulus grandis to 50% WSF No. 2 fuel oil (4.4 ppm total hydrocarbons} and found that no eggs hatched.

In contrast, Leung and Bulkley (1979) exposed embryos of Oryzias latipes to the WSF of Wyoming (Birch Creek Field) crude for 96 hours (65 to 155 milliliters WSF/liter) and reported that hatching success was unaffected. Hedtke and Puglisi (1980) also reported that hatching success of Jordanella floridae embryos was not affected by exposure to the WSF of waste crankcase oil at concentrations ranging between 0.03 to 1.0% (by volume) of the WSF.

Kuhnhold (1974) reported LC50's for young (5 to 30 hours) and old (9.5 days) embryos of Gadus morhua at 0.1 ppm and 44 ppm, respectively,

following exposure to the WSF of Venezuelan, Iranian, and Libyan crude oils (Table 1). Linden (1976, 1978) exposed fertilized eggs of Baltic herring to the WSF of Venezuelan crude at concentrations between 3.3 and 11.9 ppm, and reported that hatching success decreased linearly with exposure to increasing crude oil concentrations. Linden (1978) found that static exposure of Baltic herring to WSF No. 1 fuel oil at concentrations between 3.1 and 8.9 ppm reduced hatching success to 70%.

Smith and Cameron (1979) exposed embryos of Pacific herring to the WSF of Prudhoe Bay Crude Oil (PBCO) for 6 days at 1 ppm and reported that no eggs hatched. In a subsequent study, Cameron and Smith (1980) found that Pacific herring embryos exposed to 0.7 ppm WSF of PBCO for 48 hours exhibited significantly decreased hatching success compared with controls. Mortality of Pacific herring embryos was 100% following exposure to the WSF of PCBO (0.7 ppm) for 144 hours (6 days).

Struhsaker et al. (1974) reported the 96 hour LC50 for benzene-exposed Pacific herring embryos to be 45 ppm. Hatching success of herring embryos was significantly reduced at exposure to 45.0 ppm benzene, but was not greatly affected by the duration of exposure. Struhsaker et al. also exposed northern anchovy embryos to benzene at concentrations between 0.4 and 40.0 ppm for periods of 24 and 48 hours. Survival of embryos was reported to be lowest at a benzene concentration of 4.7 ppm following exposure for 24 hours.

Sublethal Effects

Numerous sublethal effects have been reported on fish eggs exposed to petroleum hydrocarbons. These include: 1) both delayed and accelerated development; 2) abnormal movements/behavior of developing embryos; 3) altered heart beat rate; 4) structural abnormalities of hatched larvae; 5) increased mortality of hatched larvae; 6) reduced growth of developing embryos; and 7) bioaccumulation of hydrocarbons by embryos.

Ernst et al. (1977) reported that embryos of Fundulus grandis exposed to a 12.5% WSF (1.1 ppm total hydrocarbons [TH]) of No. 2 fuel oil developed more rapidly and hatched earlier than controls. Although eggs hatched at 25% WSF (2.2 ppm) there were abnormalities of the liver, kidney, lens, and epithelial tissues in many larvae.

Anderson et al . (1977) found that embryos of F. heteroclitus and Cyprinodon variegates experienced significant reductions in heart beat rate when continuously exposed to 50% WSF of No. 2 fuel oil (10 ppm TH; 2 ppm total naphthalene (TN). At 100% WSF the heart beat rate was depressed even further and eventually resulted in death. In contrast, the heart beat rate of Fundulus similus embryos exposed to 10%, 30%, and 50% WSF of south Louisiana crude (20 ppm TH; 0.3 ppm TN) was not affected. At WSF of 70 to 100%, however, the heart beat rate was depressed from day 4 after fertilization until hatching in surviving embryos.

Sharp et al . (1979) exposed embryos of Fundulus heteroclitus to the WSF of No. 2 fuel oil and found that hatching was delayed at all exposure levels. Further, the heartbeat rate of embryos was significantly reduced at 25% WSF. Following exposure of embryos to 10%, 20%, and 25% WSF of No. 2 fuel oil larvae from successful hatchings exhibited survivorship rates ranging from 88% (10% WSF) to 0% (25%). Sharp et al also reported that embryos exposed to the 25% WSF had a peak tissue concentration (i.e. body burden) of approximately 78 ppm total naphthalenes following a 9-day exposure period. The biomagnification factor for this 9-day exposure period was 137.

Leung and Bulkley (1979) exposed eggs of Oryzias latipes to four concentrations of the WSF of Wyoming crude oil for 96 hours and found the average length of larvae at hatching was significantly less for controls. At the highest concentration (115 milliliters WSF/liter) hatching was premature for exposure periods of 24 and 48 hours. Eight day-old larvae exposed to the highest WSF concentration exhibited accelerated opercular movements. Leung and Bulkley suggested that

premature hatching was caused by early rupture of special hatching glands due to increased opercular and respiratory activity.

Kuhnhold (1974) reported that exposure of Gadus morhua eggs to the WSF of Venezuelan, Iranian, and Libyan crude oils resulted in embryonic abnormalities at gastrulation and retarded hatching for periods up to 4 to 5 days. Linden (1978) examined the effects of the WSF of No. 1 fuel oil, and Russian and Venezuelan crudes on Baltic herring eggs and noted that embryonic heart beat rates were significantly depressed at fuel oil and crude oil concentrations of 3.1 to 8.9 ppm (total hydrocarbons) and 3.3 to 11.9 ppm (total hydrocarbons), respectively. Exposure at these concentrations also resulted in delayed hatching of herring eggs, as well as a high rate (70 to 100%) of embryonic malformations. Exposure to concentrations ranging from 5.4 to 5.8 ppm (total hydrocarbons) resulted in significantly reduced lengths of newly hatched larvae. In general, Linden found that the WSF of the refined oil (No. 1) produced significantly greater effects on embryos than did the WSF of crude oils. Linden (1976) has reported similar effects on Baltic herring embryos following exposure to the WSF of Venezuelan crude at concentrations ranging from 0.1 to 10.0 milliliters oil/liter water. In particular, the heart beat rate of embryos was depressed and the frequency of abnormalities increased with increasing exposure concentrations of the WSF. In addition, larvae hatching from oil exposed eggs were significantly reduced in size.

Smith and Cameron (1979) observed that Pacific herring embryos had a significantly higher frequency of gross morphological abnormalities (primarily bent spines) when exposed to the WSF of PBCO at 1 ppm (1 microgram/gH_2O) for a period of 2 days. Electronmicrograph (EM) observations revealed that mouths and pectoral fins of embryos were also malformed. Exposure of embryos for periods exceeding 12 hours led to a significant reduction in the size of newly hatched larvae. Cameron and Smith (1980) exposed embryos of Pacific herring to the WSF of PBCO at a concentration of 0.7 micrograms HC/liter for periods of 4 to 144 hours

and found no gross abnormal i ties in newly hatched 1 arvae. EM observations, however, indicated that mitochrondria in muscle tissue were abnormal and that both muscle and brain tissue had large numbers of intracellular spaces. It was suspected that changes in mitochondrial structure and function may have affected respiration and metabolic rates, resulting in both glycogen and lipid depletion.

Eldredge et al. (1977) found that growth of benzene-exposed embryos of Pacific herring was retarded when compared with controls. The effects of benzene exposure on the oxygen consumption of embryos, however, varied depending upon the benzene concentration. Exposure to high benzene concentrations (ea. 2.1 microliter/liter) increased oxygen consumption, whereas exposure to lower concentrations (ea. 0.04 ml/1) reduced 0_2 consumption.

Struhsaker et al. (1974) reported that embryos of Pacific herring exhibited retarded development and irregular heart beat rates when exposed to benzene at concentrations of 35 to 45 ppm. Similarly, the frequency of embryonic abnormalities was highest at a benzene exposure concentration of 45 ppm. Newly hatched larvae from oil exposed eggs suffered no increased mortality following exposure to 4.8 ppm benzene; however, survival was reduced at concentrations of 17.7 and 45.0 ppm. Eldridge et al. (1978) investigated the uptake of ^{14}C-benzene by Pacific herring embryos and observed that benzene was accumulated in direct proportion to the initial exposure concentration. Equilibrium concentrations of benzene in eggs occurred 6 to 12 hours following the initial exposure. Herring eggs concentrated benzene to a level 10.9 times that of the initial exposure concentration.

Struhsaker et al. (1974) also exposed embryos of northern anchovy to benzene, and found that development was accelerated at 4.7 ppm benzene and retarded at 10.5 and 24.0 ppm benzene. At 53.5 ppm, surviving larvae were inactive and development was greatly delayed. At benzene concentrations <10 ppm the rate of yolk absorption was accelerated when compared

with controls. At concentrations of 24, and 40 to 55 ppm, however, yolk absorption was delayed. The pattern of yolk absorption was also reflected in the size of newly hatched larvae from benzene-exposed eggs. For example, larvae hatched from anchovy eggs exposed to 10.5 ppm benzene were larger than larvae produced by eggs exposed to either higher or lower benzene concentrations. This presumably occurred because embryos were less active, and therefore energy derived from yolk absorption was channeled into growth rather than activity.

LARVAL STUDIES

Lethal Effects ,

No studies were reviewed that dealt with the mortality rate of oil exposed larval fish.

Sublethal Effects

Sharp et al. (1979) reported that 10 day-old larvae of Fundulus heteroclitus exhibited an increased respiratory rate with increasing duration of exposure to the 25% WSF concentration of No. 2 fuel oil. Larval Baltic herring (24 hours old) were found to behave abnormally when exposed to the WSF of Venezuelan crude oil at concentrations of 50 to 1000 microliters/liter (Linden 1975). This abnormal behavior was characterized by short, vigorous swimming to the surface and slow sinking to the bottom. Larvae exposed to crude oil also were reported to suffer from deformed bodies with abnormal flexures.

Eldridge et al. (1977) investigated the effects of benzene exposure on yolk-sac and post-yolk-sac larvae of Pacific herring. Feeding larvae (post-yolk-sac larvae) exposed to relatively low benzene concentrations (0.4 microliter/liter) exhibited significantly higher growth and assimilation rates. ,Eldridge et al. (1978) exposed larvae of Pacific herring to ^{14}C-benzene and found that benzene was accumulated in direct proportion

to the initial exposure concentration; however, an equilibrium tissue concentration was achieved after ca. 6 to 12 hours. Feeding larvae exposed to benzene both through water and live food accumulated benzene initially from water and secondarily from food. Yolk-sac and feeding larvae accumulated up to 6.9 and 3.9 times the initial benzene exposure concentration, respectively. Pacific herring exposed to benzene (6.7 to 31.9 ppm) just following yolk absorption immediately reacted with erratic swimming and violent contractions. Subsequently, however, surviving larvae regained normal swimming and feeding behavior. Exposure of larvae to benzene produced few morphological abnormalities; however, development was delayed approximately one day. Larvae exposed to benzene concentrations of 6.7 and 12.1 ppm for a period of 48 hours exhibited significantly retarded growth rates. Three-day old larvae exposed to benzene concentrations of 13.0 and 39.1 ppm had significantly increased respiratory rates when compared with controls.

JUVENILES/ADULTS

Lethal Effects

Anderson et al. (1974) performed 96 hour toxicity tests (LC50s) on Cyprinodon variegates, Menidia beryllina, and Fundulus similus using the WSF of south Louisiana crude, Kuwait crude, No. 2 fuel oil, and Bunker C residual oil (Table 1). In general, M. beryllina was the most sensitive species tested, and refined hydrocarbon products were most toxic. Moles et al. (1979) determined the median tolerance 1 imits (TL_ms) of three-spine sticklebacks, slimy sculpin, dolly varden, and Arctic grayling to the WSF of Prudhoe Bay crude oil (PBCO) and benzene. The test species were most sensitive to PBCO, with TL_ms ranging from 2.7 ppm for dolly varden to >10.5 ppm for three-spine sticklebacks (Table 1). All species were less sensitive to benzene; however, the relative sensitivities of the four species were the same as for PBCO (Table 1).

Rice et al. (1976) reported 24 and 96 hour TL_ms for pink salmon, dol" y varden, cod, and tubesnout exposed to Cook Inlet crude oil (CICO)

and No. 2 fuel oil. The 24 hour TL_ms for CICO ranged between 2.48 ppm (cod) and 4.13 ppm (pink salmon). At 96 hours, the TL_ms ranged from 1.34 ppm for tubesnout to 2.94 ppm for dolly varden. In general, the test species exhibited lower tolerances to CICO at 96 hours, as well as less variability among species. The 24 hour TL_ms for No. 2 fuel oil ranged from 0.89 ppm for pink salmon to >4.56 ppm for cod. Similarly, the 96 hour TL_ms ranged from 0.81 ppm for pink salmon to 2.93 ppm for cod. For both exposure periods, pink salmon were most sensitive and cod were least sensitive to No. 2 fuel oil.

Hedtke and Puglisi (1980) exposed juvenile flagfish to the WSF of waste crankcase oil and reported 96 hour LC50s of 36,200 microliter WSF/liter and 9,500 microliter WSF/liter for static and flow-through system tests, respectively (Table 1). Similar tests with an oil/water dispersion (OWD) resulted in 96 hour LC50s of 485 microliters WSF/liter and 82.7 microliter WSF/liter for static and flow-through system tests. Morrow (1973) determined mortality rates of juvenile coho and sockeye salmon exposed to PBCO for 72 and 96 hours. At a concentration of 3,500 ppm PBCO, coho mortalities ranged from 42 to 50% at 72 hours to 56 to 62% at 96 hours. Sockeye mortalities for exposure to PBCO for 96 hours ranged from 6.7% (1,750 ppm exposure) to 44.8% (1,000 ppm exposure; Table 1).

Kern et al. (1979) determined median tolerance limits (96 hours) for juvenile pink salmon exposed to the WSF of CICO, toluene and naphthalene at temperatures of 4°, 8°, and 12°C. Tolerance limits ranged from 1.37 ppm (naphthalene) to 6.41 (toluene), 1.69 ppm (CICO) to 7.63 ppm (toluene) and 1.24 ppm (naphthalene) to 8.09 ppm (toluene) at 4°, 8°, and 12°C, respectively (Table 1). The juvenile salmon were generally most sensitive to CICO and naphthalene and least sensitive to toluene. The sensitivity of pink salmon to CICO and toluene increased with decreasing temperatures.

Moles (1980) reported 96 hour LC50s for parasitized and non-parasitized juvenile coho salmon when exposed to PBCO, toluene, and

naphtha lene. For non-parasitized fish, LC50s ranged from 3.22 ppm (naphthalene) to 10.38 ppm (PBCO) (Table 1). Parasitized fishes were much more sensitive to hydrocarbon exposure, with LC50s ranging between 0.77 ppm (naphthalene) and 3.08 ppm (toluene). Prior to conducting sublethal effects studies, Rice et al. (1977) determined the acute toxicity of juvenile pink salmon to the WSF of CICO, PBCO, and No. 2 fuel oil. Median tolerance limits for 24 and 96 hours, respectively, ranged from 0.89 ppm (No. 2 fuel oil) to 4.13 ppm (CICO) and 0.80 ppm (No. 2 fuel oil) to 2.90 ppm (CICO). Number 2 fuel oil was most toxic and CICO least toxic hydrocarbon at the termination of both 24 and 96 hour exposure periods; however, pink salmon were more sensitive after 96 hours.

Benville and Kern (1977) reported the acute toxicity of six monocyclic aromatic crude oil components to striped bass. Twenty-four hour and 96 hour LC50 test results were very similar (Table 1) with LC50s ranging between 2.0 microliters/liter (p-xylene) to 11.0 microliter/liter (o-xylene). The most toxic crude oil component was p-xylene and the least was o-xylene. Meyerhoff (1975) reported the 96 hour LC50 for juvenile striped bass exposed to benzene as 10.9 microliters/liter (ppm).

Sublethal Effects

Thomas and Rice (1975) exposed juvenile pink salmon to the WSF of Prudhoe Bay crude oil at concentrations ranging from 0.015 to 3.46 ppm total hydrocarbons. At concentrations exceeding 2.83 ppm, opercular rates were increased significantly for as long as 9 to 12 hours after exposure. Opercular rates increased proportionally with increasing exposure dose. Thomas and Rice (1979) reported that juvenile pink salmon exposed to sublethal concentrations of toluene (1.6 to 5.1 ppm) and naphthalene (0.4 to 0.98 ppm) for 10 hours experienced elevated opercular and oxygen consumption rates immediately following-exposure. Maximum opercular and oxygen consumption rates occurred at 2 to 4 and 6 to 8 hours, respectively, following initial exposure; however, both parameters declined after exposure was terminated. Juvenile pink salmon were exposed to the

WSF of Cook Inlet crude (C ICO), Prudhoe Bay crude (PBCO), and No. 2 fuel oil by Rice et al. (1977) for periods up to 22 hours. The opercular rate of pink salmon was elevated following exposure to all three hydrocarbons, with the maximum rate occurring 3 to 6 hours after initial exposure. Pink salmon were most sensitive to No. 2 fuel oil ($\geq$0.24 ppm) followed by PBCO (>1.03 ppm), and CICO (>1.95 ppm).

Stegeman and Sabo (1976) exposed adult Fundulus heteroclitus and Stenotomus versicolor to No. 2 fuel oil (180 to 200 micrograms total hydrocarbon/liter) and found that lipid metabolism was altered in both species. There was a net decline in lipogenesis in the liver of both species (at 180 to 200 ppb total hydrocarbon), and low lipogenesis in the gill, muscle, and brain tissue of Fundulus heteroclitus. In a later study, Sabo and Stegeman (1977 found that liver metabolism of Fundulus heteroclitus was greatly affected by exposure to No. 2 fuel oil (180 ppb) for 8 days. Although glucose metabolism was not affected, acetate metabolism was reduced in oil exposed fishes. In addition, phospholipids, fatty acids, and triglycerides decreased in concentration and cholesterol increased.

Fletcher et al. (1979) found that the blood plasma copper (Cu^{+2}) concentration of cunner an Atlantic wrasse (Tautogolabrus adspersus) was significantly reduced after exposure to sublethal concentrations of Venezuelan crude oil for a period of 6 months. All other blood parameters tested (Ca^{+2}, Mg^{+2}, Zn^{+2}, and total proteins) were not significantly different between oil exposed and control fishes.

Kristofferson et al. (1973) exposed juvenile pike to a sublethal concentration of phenol (5 ppm) for 7 days and found no differences in hemoglobin, hematocrit, total plasma, Na^{+}, K+, CA^{+2}, Mg^{+2}, or Cl^{-} between oil exposed fishes and controls. Cunner were exposed to sublethal concentrations of Venezuelan crude for two weeks (Kiceniuk et al. 1980), resulting in elevated blood plasma hematocrit, increased gall bladder size, and decreased spleen weight. Payne et al. (1978) found that

plasma chloride and testes weight of adult cunner were depressed after a 6 month exposure to Venezuelan crude. No significant histopathological changes were found in the liver, kidney, heart, spleen, gonad, gill, muscle, or gut tissue of oil exposed individuals.

Juvenile striped bass exposed to sublethal concentrations of benzene (3.5 and 6.0 microliter/liter) for 4 weeks were found to have significantly reduced wet and dry body weights and percent body fat (Kern et al. 1976a) when compared with controls. Benzene exposure also induced hyperactivity and decreased feeding success (i.e. smaller ration); probably through the impairment of food location. McCain et al. (1978) reported a weight loss in English sole exposed to oil contaminated sediments.

Hedtke and Puglisi (1980) performed life cycle bioassays on Jordanella floridae and reported that egg production per female was significantly reduced at exposure concentrations exceeding 3,380 microliter WSF/liter.

Walton et al. (1978) exposed cunner to a sublethal concentration of Venezuelan medium crude (1 to 2 ppm) and found that aryl hydrocarbon hydroxylase (AHH) activity was increased 2 to 6 times above uninduced levels. Elevated AHH levels decreased greatly less than 7 days following the end of exposure. Feeding of crude oil or crude oil-contaminated food (mussels) at a concentration of 500 mg/kg body weight produced a 5-fold induction in AHH levels within 2 weeks.

Payne and Penrose (1975) reported that livers of adult brown trout and capelin exhibited significantly elevated AHH activity when exposed to Venezuelan crude (1.0 ppm) for periods of 7 to 17 days. Payne (1977) also found that rainbow trout exposed to low concentrations of Venezuelan crude produced significant increases in AHH activity. Gruger et al. (1977) exposed juvenile coho salmon to the WSF of Prudhoe Bay crude for 6 days at 15 and 150 ppb, and observed that AHH activity was

significantly increased. Kurelec et al. (1977) reported that benzo[a]-pyrene monooxygenase (BPMO) was induced in Blennius pavo after exposure to 170 ppb and saturated diesel No. 2 oil. At 170 ppb, the induction response was delayed until day 14, whereas under saturated conditions the response occurred on the third day. BPMO activity was maintained at high levels for at least one month following transfer to non-contaminated water.

Neff et al. (1976) exposed Fundulus similus to the WSF of No. 2 fuel oil (2 ppm total naphthalenes) for 2 hours and then followed deputation of hydrocarbons for 366 hours. All organs examined (e.g. gut, liver, gall bladder, gills, heart, brain, and muscle) rapidly accumulated naphthalenes within the 2-hour exposure period, with the gall bladder (2,300 ppm) and brain (620 ppm) accumulating the highest concentrations. Release of naphthalenes began immediately following transfer to fresh water, reaching undetectable levels after 366 hours.

Lee et al. (1972) studied the uptake, metabolism, and deputation of ^{14}C-naphthalene and ^{3}H-3,4 benzopyrene by Gillichthys mirabilis, Oligocottus maculosus, and Citharichthys stigmaeus. Both label led hydrocarbons were taken up by fishes via the gills, metabolized in the liver, transferred to the bile, and then excreted in the urine. The major metabolizes of radiolabelled benzopyrene and naphthalene were 7,8 dihydro-7,8-dihydroxy-benzopyrene and 1,2-dihydro-1,2 dihydroxynaphthalene.

Roubal et al. (1978) exposed both coho salmon and starry flounder to the WSF of Prudhoe Bay crude (0.9 ppm) for a 2 to 6 week period. The highest accumulation of hydrocarbons in coho salmon occurred in the muscle, with the maximum bioconcentration occurring after 5 weeks. All hydrocarbons were depurated within l-week following termination of exposure. Similarly, the highest accumulation of hydrocarbons by starry flounder occurred in muscle tissue within 1 week following initial exposure. Relatively high bioconcentration factors also occurred in the

liver and gill tissue. After removal of oil-exposed individuals to non-contaminated water for a period of 2 weeks, the hydrocarbon level remained elevated in muscle tissue, but was near normal levels in the liver and gill tissues.

McCain et al. (1978) reported that English sole exposed to North Slope crude oil contaminated sediments (700 micrograms/gram dry weight) for 4 months readily accumulated aromatic and alkane hydrocarbons in the skin, muscle, and liver. 1- and 2-methynaphthalene and 1,2,3,4-tetramethyl benzene were accumulated to a greater extent than other oil components. Tissue burdens of hydrocarbons decreased with increasing exposure time, such that after 27 days of exposure, only the liver had a detectable hydrocarbon burden. The authors' suggested that induction of AHH activity eventually resulted in hydrocarbon deputation by English sole.

Kern et al. (1977) examined the uptake, tissue distribution, and deputation of ^{14}C-benzene and ^{14}C-toluene by Pacific herring following a single 48-hour dose at 100 ppb. Toluene accumulated in higher concentrations in all tissues than did benzene. The highest concentration of both hydrocarbons accumulated in the gall bladder and the lowest accumulated in the gonads. The maximum tissue concentrations of both compounds *were* reached within 24 to 48 hours, with toluene accumulating somewhat more rapidly. Deputation of both compounds was rapid, with tissue concentrations at non-detectable levels 1 to 2 days following exposure termination. The gall bladder, intestine, and pyloric caeca retained hydrocarbon residues for the longest period following termination of exposure.

Kern et al. (1976b) also investigated the uptake, distribution, and deputation of ^{14}C-benzene by striped bass and northern anchovy. Striped bass and northern anchovy *were* exposed to 0.09 and 0.007 to 3.7 microliter/liter ^{14}C-benzene, respectively, for 48 hours. Both species exhibited similar patterns of benzene accumulation, with the highest

concentrations in the gall baldder, mesenteric fat, colon, intestine, and liver. The highest rate of intake occurred in the first 6 hours, and maximum tissue concentrations were obtained within 4 days. Tissue burdens of benzene were higher in northern anchovy than striped bass. Benzene residues were rapidly depurated after cessation of exposure, with the gall bladder, mesenteric fat, liver, and gill tissues retaining residues for the longest period.

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